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Testing the capacity of the National Biological Dose Response Plan (NBDRP) EX40801

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Testing the capacity of the NBDRP – EX40801

Introduction

The National Biological Dose Response Plan (NBDRP) is currently comprised of four core laboratories (Health Canada (HC), Defence Research and Development Canada–Ottawa (DRDC), McMaster University (MU), and Atomic Energy of Canada Limited (AECL)) that are capable of providing radiation biological dose estimates using the dicentric chromosome assay (DCA).

As indicated in the CRTI-06-0146RD charter, the existing biological dosimetry capacity in Canada will be greatly enhanced by conducting ongoing training and annual exercising of the four core laboratories. It was also stated that the NBDRP will expand linkages with our U.S. counterparts to work towards an integrated North American emergency response network. To this end, this exercise was designed to:

1) test the time required for each laboratory to provide biological dose estimates on 10 irradiated and blinded samples.
2) validate the scoring capacity and capabilities of the NBDRP core laboratories.
3) further investigate the advantages of the addition of an alternate scoring protocol (QuickScan) and/or biodosimetry method, the cytokinesis block micronucleus assay (CBMN), to our biodosimetry plan in order to expedite dose estimates or assist in sample prioritization.
4) expand the exercise to include two U.S. laboratories (Armed Forces Radiobiology Research Institute (AFRRI) and Oak Ridge Institute for Science and Education (ORISE)) who could be incorporated into our Network.

To address the third goal, the four core laboratories (HC, DRDC, AECL, MU) plus ORISE performed the QuickScan method and HC, DRDC and AECL performed the CBMN on the exercise samples as a follow up to the initial dose estimates using the standard triage DCA.

Finally, to ensure QA/QC and privacy of donor information, all samples were bar-coded in the current exercise. This allowed laboratories to test their ability to efficiently utilize their barcode readers in a simulated emergency and eventually allow incorporation of bar-coded samples into the DCA standard operating procedure for the NBDRP.

Scenario

The exercise was initiated on November 3, 2008. The scenario for over-exposure involved 60 people potentially being exposed.

Blood Collection

All donors were volunteers that willingly responded to an advertising call for participation in a research proposal approved by the HC Research Ethics Board. In total 60 blood samples were collected. Blood was drawn into six 4mL lithium–heparinized Vacutainer® tubes from each of 10 individuals at HC.

In Vitro Irradiation of Blood Samples

Once all 60 samples were collected, the samples were randomly irradiated at 10 different doses so that the six blood tubes from one donor received the same dose and the blood from each donor received a different dose (Table 1). Each laboratory received matching samples, one from each donor/dose. The samples were blinded so the dose received could not be identified. Each sample was irradiated with a dose between 0 and 4 Gy with ^{137}Cs using a Gammacell 40 at a dose rate of 0.81 Gy/min. The irradiation and sample blinding were done by a third party.

Table 1. Blood collection and irradiation

Sample #	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
Donor #	1	2	3	4	5	6	7	8	9	10
# of 4mL tubes taken	6	6	6	6	6	6	6	6	6	6
Dose (Gy)	0.5	1.8	1.5	1.2	2.0	0.0	0.2	1.0	3.0	2.5

Communications with the NBDRP Network Laboratories

On receiving a call from the person acting as a physician in this exercise, HC called the other three core laboratories, along with the two participating US laboratories, to inform them of the accident and to prepare them for receiving the samples.

Transportation of Blood Samples

In a real scenario, the first contact laboratory would provide the instructions (Annex A) for collecting and shipping blood samples. In this exercise scenario, instructions were sent to the acting physician although the samples were collected at HC and distributed to the remaining five laboratories. The samples were shipped to all laboratories (except DRDC) by FedEx Express and were received the following day. DRDC picked up their samples at HC and started culturing the same day. An instruction form was sent with each shipment (Annex B)

Analysis of Blood Samples

Each of the six laboratories processed their 10 samples and reported back to HC where the results were compiled. All 18 trained scorers from the participating laboratories analysed all 10 samples. For full DCA triage, each scorer analyzed 50 cells or 30 dicentrics, ensuring that each cell had 46 centromeres. Seventeen of the scorers across the laboratories also used the "QuickScan" method devised by AECL, in which individual chromosomes are not counted but the whole cell is examined for damage[1]. Three laboratories (HC, DRDC and AECL) also analysed the samples using the cytokinesis block micronucleus assay (CBMN)[2]. For each method, total scoring time was tabulated.

Results

The results from DCA triage scoring at each laboratory are shown in Figure 1 along with solid lines indicating the ±0.5 Gy range. Each symbol represents the result from one scorer analysing 50 cells (30 dicentrics). Scorers from the same laboratory are shown in the same colour. In Figure 2, the dose estimates based on QuickScan are shown. Figure 3 shows the doses determined using the CBMN assay. Scoring using both full triage and QuickScan was also analysed after 10, 20 and 50 cells; these results are summarized along with the CBMN results in Table 2. The scoring time for each method is shown in Table 3.

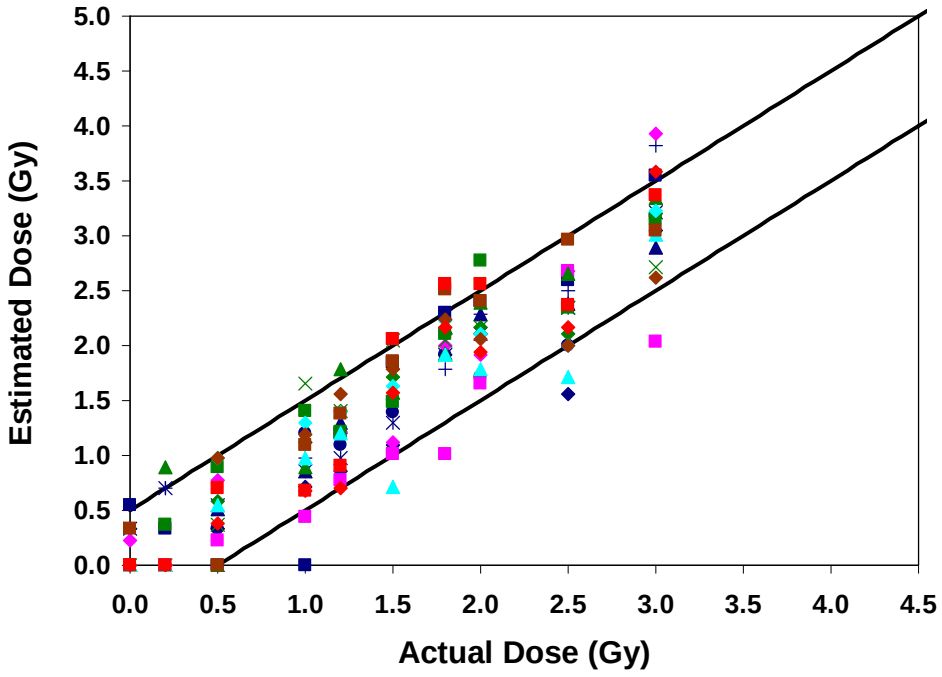

Figure 1: Dose estimates derived after scoring 50 cells with full triage scoring.

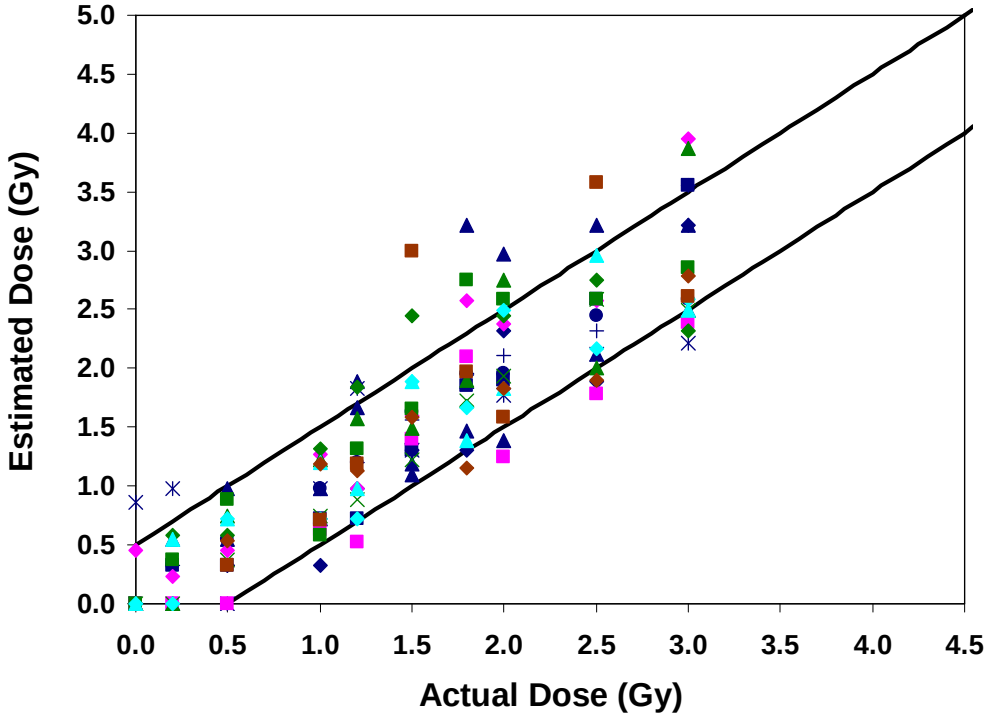

Figure 2: Dose estimates derived after scoring 50 cells using the QuickScan criteria.

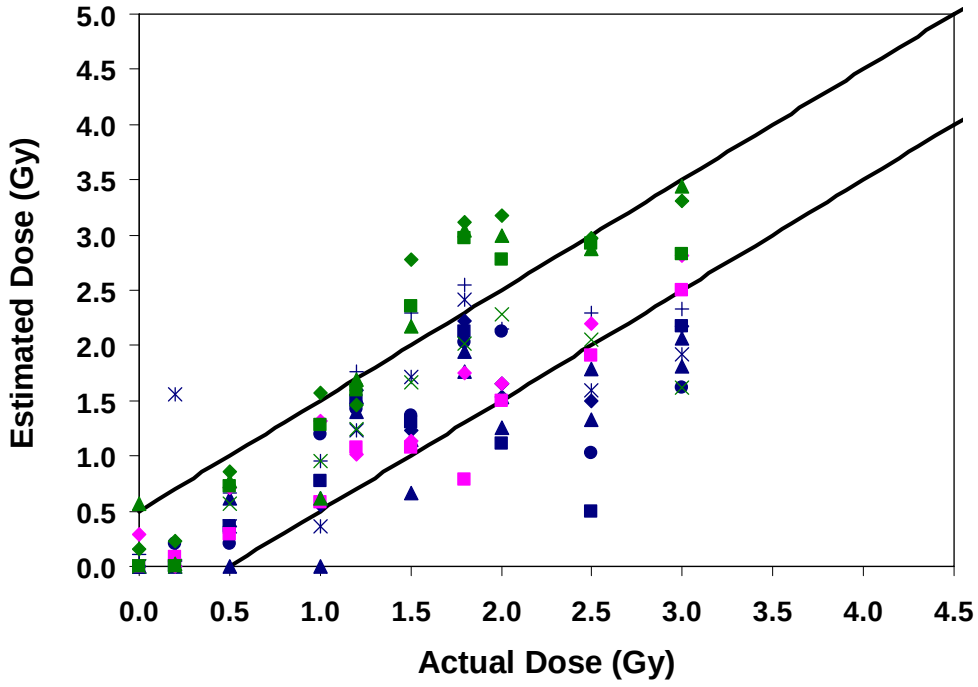

Figure 3: Dose estimates derived after scoring 200 BNC using the CBMN Assay.

Table 2. Comparison of scoring methods

Method	% within 0.5 Gy	% over estimates	% underestimates	>1 Gy not identified as exposed (%)
Full Triage: 50 spreads	88.3	7.2	4.5	0.6
20 spreads	77.8	9.4	12.8	4.4
10 spreads	67.8	15	17.2	10.6
QuickScan: 50 spreads	82.3	11.2	6.5	0.0
20 spreads	73.0	12.9	14.1	3.5
10 spreads	62.9	17.7	19.4	8.8
CBMN (200 BN cells)	71.5	12.3	16.2	0.8

Table 3. Scoring time for Full Triage vs. QuickScan

Method	Ave. time to score 10 slides
Full Triage	760 minutes
QuickScan	196 minutes
CBMN (200 BN cells)	129 minutes

To highlight the progress made over the past year, a comparison of the results from the exercises EX30701 and EX40801 is shown in Table 4.

Table 4. Summary of EX30701 and EX40801

Method	Equivalent # of Samples		% within 0.5 Gy		Scoring Time per Sample (min)	
	EX07	**EX08**	**EX07**	**EX08**	**EX07**	**EX08**
Triage DCA	150	180	83	88	126	76
QuickScan DCA	90	170	80	82	20	20
CBMN	110	130	57	71	13	13

Discussion

Ten blinded, irradiated samples were sent to each of the four reference laboratories of the National Biological Dose Response Plan plus two US laboratories. Samples were scored for the dicentric chromosome assay and the CBMN assay. Using the DCA, cells were analysed for either 50 cells or 30 dicentrics (and rings) according to Full Triage biological dosimetry standard or QuickScan scoring. Dose estimates were also determined after scoring 10 and 20 cells. The results of the full triage method were the most accurate with 88% of the dose estimates falling within 0.5 Gy of the delivered dose. Not only was this a higher success rate than in our last exercise (83%), but a greater number of scorers participated and were tested for their accuracy, including new staff with less experience. For comparison, in the previous exercise, 15 scorers each scored the same 10 samples for an equivalent of 150 samples being scored whereas in this exercise, the equivalent of 180 samples were scored by standard triage DCA, demonstrating an increased capacity of the network.

As a strategy to increase the throughput for biological dosimetry using the DCA, decreasing the number of cells analysed has been considered. Decreasing the number of cells scored, but still ensuring the presence of 46 centromeres, reduced the scoring time but also the accuracy. Scoring 20 cells reduced the accuracy to 78% whereas scoring only 10 cells resulted in an even greater loss of accuracy (68% within 0.5 Gy).

The QuickScan method devised by AECL, however, greatly increased the speed, reducing the average time to analyze 10 samples from 760 minutes to 196 minutes while maintaining a high level of accuracy (82% within 0.5 Gy). With this method it was observed that the high dose samples were very quick to score although inherently less accurate compared to the DCA since 5 dicentrics were found in few cells. The samples that took the longest to score were the very low doses. By using the QuickScan method but stopping after 10 spreads if no damage was found resulted in an additional reduction in scoring time but also a loss in accuracy.

The CBMN assay was introduced into our exercise as a pilot study in 2007 as a possible screening tool in situations where large sample volumes are expected. While not radiation-specific, this assay is radiation-responsive and could provide a useful tool to screen out samples which did not receive a dose, and identify high priority samples for full DCA analysis. This assay was included again this year with the results shown in Figure 4. Although the CBMN only yielded 71% of dose-estimates within 0.5 Gy of the actual dose, this was a substantial improvement over the previous exercise where only 57% of the dose estimates were accurate. This is likely due to an increased experience in scoring among the participating laboratories. Furthermore, most of the outliers were from one laboratory where 15 mL blood cultures were used (rather than the standard 10 mL blood culture), to generate more slides since this lab had a larger number of scorers. Subsequent analysis has indicated that using a higher blood culture volume, without adapting the hypotonic and “fixation” conditions (i.e. volumes) results in poor slide quality and a reduced rate of MN detection in highly exposed samples (data available upon request). As such, it is expected that a much higher proportion of ‘accurate’ dose-estimates will be realized in future exercises using the CBMN assay, now that this source of error has been identified. As indicated in Table 3, the CBMN assay is considerably more beneficial in relation to scoring time for each sample relative to the DCA assay.

An important observation for all three assays is that for 50 cell scoring for DCA and QuickScan and 200 cell scoring for CBMN, the percentage of samples receiving more that 1 Gy that were not identified as exposed was less than 1 %. As medical intervention would only be considered for individuals receiving more than 1 Gy, these assays would provide an extremely low false negative rate.

Conclusions

Based on this exercise, it is recommended that for a pre-triage screening of large sample numbers, the QuickScan method and/or the CBMN assay be used to quickly prioritize samples for further, more accurate analysis. Using these methods, and the full capacity of the network, it is feasible to produce initial dose estimates for 170 individuals within a few hours of the samples being processed. With the initial processing time, initial dose estimates would be available within 4 days of sample collection.

Overall, this exercise demonstrated an increased capacity for performing the DCA and CBMN for biological dosimetry, not only through an increased number of qualified scorers but also through new scoring strategies. It also demonstrated the operability of the network and its ability to provide timely dose estimates for a large number of exposed individuals. This exercise also demonstrated the feasibility of involving laboratories from the US to assist in biological dosimetry when our country's capacity is overwhelmed.

Lessons Learned

1. Blood shipping:

- Develop a check list of items to be included in each box and labels required.
- Record the serial numbers of the OSL dots placed in each box.
- "Rush" or "perishable" stickers should be added to the shipping containers.
- Use Purolator (when possible) for AECL shipments as they deliver to Chalk River 2 x daily.

2. Shipping boxes back to HC:

- Do not remove "DO NOT X-RAY" and "DO NOT FREEZE" labels as the boxes still contain OSL dots and temperature loggers.

3. Sample tracking:

- Create a table to be able to keep track of shipping/receiving of samples such as:

Lab	Initial contact		Ok to Receive?	Time Blood Shipped	Tracking Number Sent	Confirmation of Tracking Number Receipt	Time Blood Received	Comments
	Person	Phone Number						

4. Barcoding:

- Printing labels still needs better organization

- Do not put labels on slides before Geimsa staining

5. Culture Volumes:

- It is essential that the 10 mL blood culture volumes are used for the CBMN assay.

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References

1. Flegal, F.N., Devantier Y., McNamee, J.P., Wilkins, R.C. QuickScan dicentric chromosome analysis for radiation biodosimetry , Health Physics Journal, In Press (2009).

2. McNamee, J.P., Flegal, F.N., Boulay Greene, H., Marro, L., Wilkins, R.C. Validation of the Cytokinesis-Block Micronucleus (CBMN) assay for use as a triage biological dosimetry tool. Radiation Protection Dosimetry, In Press (2009).

Annex A: Blood Sample Collection and Shipping

- Analysis of chromosomal aberrations in human peripheral blood lymphocytes is the present day standard for the biological assessment of radiation exposure. To optimize the recovery of lymphocytes from the blood, it is very important that the blood be collected and shipped according to the protocol outlined below.
- Before blood samples are taken please notify HC so that we can prepare for arrival and pick up.
- All blood samples are to be collected into lithium heparin tubes (if not available sodium heparin tubes are acceptable), and are to contain at least 3 mL (ideally 2 x 5 mL tubes). Gently rock the tubes to ensure proper mixing. Label the tubes unambiguously using the coding system identified by the receiving laboratory.
- Package the blood sample carefully to prevent breakage of the tubes in transit. The blood should be maintained at approximately 20°C. **Blood samples must not be frozen.** One method of maintaining blood at room temperature is to place the tubes on a gel pack that has been allowed to stay at room temperature for several hours.
- Immediately following blood collection, ship the samples by **special transportation and use overnight air express so we can receive the blood early in the morning following sample collection.** Contact the laboratory to confirm the shipment and inform us of the **Way Bill** number. THIS IS IMPORTANT FOR TRACKING THE SAMPLES.
- For best results blood must be received within 24 h of sampling.
- For air transport, packaging and labelling should conform to the current International Air Transport Association (IATA) regulations. These require that blood samples be packed to conform to **United Nations Regulation 650** for biological substances. Saf-T-Pak manufactures packaging that meets these requirements (STP 210) (www.saftpak.com). Other packaging is acceptable providing it meets the requirements stated below.
- Packaging:
  - leak proof primary container (blood collection tube)
  - leak proof secondary container (e.g. Ziploc bag)
  - absorbent material placed between the primary and the secondary container
  - if purchased must be marked with **TC-125-1B** (e.g. STP 210 packaging)
  - if the shipper is making his own packaging, it must be a rigid outer packaging, and the exterior must be marked with **125-1B**
- Marking and labelling on outer package for air transport:
  - **name, address and telephone number of receiver and shipper**
  - **name, address and telephone number of person responsible if other than shipper**
  - **Biological substances, category B**
  - diamond shaped **UN3373 label**
  - **2 orientation arrows** placed on opposite sides of the package
  - **DO NOT X-RAY, DO NOT FREEZE**
- **An itemized list** of package contents must be placed between the secondary and outer packaging
- Waybill:
  - in "Description", enter only: **UN3373 Biological substances, category B**

Ship to: Health Canada
Consumer and Clinical Radiation Protection Bureau
775 Brookfield Road, PL 6303B
Ottawa, ON K1A 0K9
Phone: (613) 355-6028 Fax: (613) 941-1734

Annex B: EX30701 Exercise **December 3rd, 2007**

Instructions for Network Laboratories

Please find enclosed 10 randomly irradiated samples for biological dosimetry using the Dicentric Chromosome Assay and Cytokinesis Block Micronucleus Assay.

The samples are coded as follows:
E3VIAL1S01
E3VIAL1S02
E3VIAL1S03
E3VIAL1S04
E3VIAL1S05
E3VIAL1S06
E3VIAL1S07
E3VIAL1S08
E3VIAL1S09
E3VIAL1S10

N.B. VIAL# is different for every lab

Results should be faxed and e-mailed back to Health Canada for compilation and a report will be sent to the CRTI Secretariat
Fax: Attention Vinita Chauhan
(613)952-7584
E-mail : Vinita_Chauhan@HC-SC.GC.CA
A follow-up e-mail will be sent with a sample reporting sheet.

If you have any questions, please feel free to call me at **(613)-355-6028 (cell)** or **(613)-941-7263 (office)**
E-mail Ruth_Wilkins@hc-sc.gc.ca

Thank you for helping in processing these samples.

Ruth Wilkins
Health Canada
Consumer and Clinical Radiation Protection Bureau
775 Brookfield Road
Ottawa, Ontario

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